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MONENSIN INCREASES CYTOSOLIC CALCIUM IN FRTL-5 CELLS

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INTRODUCTION

Calcium is thought to play an important role in stimulus-secretion coupling in secretory cells. Monensin, a Na^+-specific ionophore which raises intracellular sodium content, also increases the cytosolic free calcium concentration $[Ca^{2+}]_i$ in several cells (1,2). The objectives of the present study were (a) to examine the effect of increasing $[Na^+]_i$ on cytosolic Ca^{++} in FRTL-5 cells, and (b) to determine the relative contributions of extra- versus intracellular calcium in producing any such changes.

MATERIALS AND METHODS

Materials

Cells. FRTL-5 cells were maintained in 95% air - 5% CO_2 at 37°C. The medium used was Coon's modified Ham's F-12 supplemented with 5% calf serum, 1 mM non-essential amino acids, TSH (1 mU/ml), insulin (10 µg/ml), transferrin (5 µg/ml), glycyl-L-histidyl-L-lysine acetate (2 ng/ml), somatostatin (10 ng/ml), and cortisol (1 nmol/l). Cells were plated in petri dishes at $2x10^5$ cells/ml, fed twice weekly, and passaged every 7-8 days.

Methods

Loading of Cells. Cells were washed, resuspended at $5x10^7$ cells/ml in HBSS, and incubated with Indo-1 AM (5 µM) for one hour at 37°C in a shaking water bath. Cells were resuspended at $2.5x10^6$ cells/ml in HBSS with 0.02% BSA for study.

Fluorescence Measurements. Two ml aliquots were placed in quartz cuvettes and fluorescence recordings obtained on an SLM 8000 spectrofluorometer. The excitation wavelength was 350 nm. Emmission was recorded as the rate of intensities at 405 and 480 nm. Calculations of $[Ca^{2+}]_i$ were determined as previously reported (3).

RESULTS

Extracellular Ca^{++} Present

Baseline Calcium. Cytosolic calcium content under basal conditions averaged 220 ± 6 nM (n=50).

Monensin Effect. The addition of monensin produced a dose dependent increase in $[Ca^{2+}]_i$ (Table).

Calcium Blockers. Three classes of Ca^{++} channel blockers (nifedipine, verapamil, diltiazem) had no effect on inhibiting the monensin stimulation of $[Ca^{2+}]_i$. Similar results were obtained when TMB-8 and ryanodine were used to block the release of intracellular Ca^{++} stores.

Na^+/K^+ ATPase. Ouabain (10^{-3}M) had no effect on the monensin stimulation of $[Ca^{2+}]_i$.

Extracellular Cations Absent

Monensin Effect. In the absence of extracellular Ca^{2+}, monensin produced a negligible change in $[Ca^{2+}]_i$ (Table). Subsequent addition of ionomycin produced a release of Ca^{2+} from intracellular stores. In Na^+ free buffer, the $[Ca^{2+}]_i$ response to monensin was inhibited by 80% (Table).

TABLE: MONENSIN EFFECT ON CYTOSOLIC CALCIUM (% Change)

Monensin Dose (M)	Ca^{2+}	+	-	+
	Na^+	+	+	-
10^{-4}		420 ± 54%	6 ± 3%	79 ± 10%
10^{-6}		81 ± 11%	N.D.	15 ± 15%
10^{-8}		32 ± 6%	N.D.	N.D.

Ca^{2+} = 1.27 mM; Na^+ = 140 mM; N.D. = not tested

DISCUSSION

This study demonstrates that changes in intracellular sodium dramatically increase the cytosolic free calcium concentration in FRTL-5 cells. The source of calcium is virtually all extracellular. The calcium influx is independent of calcium channels or Na^+/K^+ ATPase activity. While the response is highly dependent upon extracellualr Na^+, a small effect of monensin was independent of Na^+.

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